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THE GERMINATION OF THE SEEDS OF
ANGIOSPERMOUS ROOT-PARASITES.

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PART III.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST FACTOR
ON THE GERMINATION OF THE SEED OF *ALECTRA*
VOGELII BENTH.

INTRODUCTION.

As pointed out previously (Botha, 1948 and 1950, I) the germination process of the seed of *Alectra Vogelii* can be divided into two consecutive phases. During the first phase, which is brought about by moist conditions and a suitable temperature, the seed becomes sensitive to the host factor; and during the second phase, for which the presence of the host factor is essential, the radicle starts growing. It has also been pointed out elsewhere (Botha, 1950, II) that the time of pre-exposure to moist conditions, the temperature of pre-exposure and the concentration of the host factor are complementary in their effect on the percentage germination. These findings are consistent with the hypothesis put forward by Brown and Edwards (1946) with regard to the germination of the seed of *Striga lutea*, viz. that during "pretreatment" (pre-exposure to moist conditions) the seed itself produces a stimulating substance which is the same as or similar to that released by the host roots. In the present paper further evidence is presented in this connection.

EXPERIMENTAL METHODS.

Four experiments were carried out. To distinguish between the four, I shall hereafter refer to them as Experiments 1, 2, 3 and 4.

In Experiments 1 and 2 aliquot lots of seed which had been pre-exposed for 10 days at 30°C. were exposed to different concentrations of the host factor for varying periods of time; and in Experiments 3 and 4 the effect of time of exposure to the host factor on the germination of aliquot lots of seed pre-exposed for different periods of time was investigated, undiluted host solution being applied. The effect of relatively long periods of exposure was examined in Experiments 1 and 3, while in Experiments 2 and 4 the time of exposure was varied over a much shorter range.

The several concentrations of the host stimulant applied in Experiments 1 and 2 were obtained by dilution of the host solution with appropriate volumes of distilled water. The concentration of the stimulant in the undiluted host solution was in each case taken to be 100 arbitrary units.

The method of exposing the seed to the host solution was as follows: Aliquot lots of the pre-exposed seed were transferred to small linen bags which were then suspended in the host solution. After the required time had elapsed, the bags were removed and thoroughly rinsed in running water. The seed was then brought into micro-culture at 30°C., drops of distilled water being used as culture medium. In all experiments aliquot lots of pre-exposed seed were also brought directly into micro-culture at 30°C., drops of the host solution being used as culture medium in this case. This latter treatment served as a control and is referred to as an exposure time of 3 days in the Tables. Exposure took place at room temperature ($\pm 18^\circ\text{C}.$) in Experiments 2 and 4 (short periods of exposure), while in Experiments 1 and 3 the solutions containing the seed were placed in the incubator at 30°C. during the time of exposure.

The details of the preparation of the host solutions and of the technique of the micro-culture method were essentially the same as those described elsewhere (Botha, 1950, I). Five replicates were involved in each germination test. Each percentage value in Tables 1, 4, 7 and 10 is, therefore, the mean of five observations. The results of all experiments were analyzed for variance.

RESULTS.

The results of Experiment 1 are presented in Tables 1—3, those of Experiment 2 in Tables 4—6, those of Experiment 3 in Tables 7—9 and those of Experiment 4 in Tables 10—12.

(1) *Experiments on the Effect of Time of Exposure in Relation to Concentration of the Host Factor.*

(a) *Experiment 1.*—From the data of this experiment it is clear that

the time of exposure as well as the concentration of the host factor has a highly significant effect on the percentage germination and that the interaction between these two factors is also highly significant. (For each of these three components $P < 0.01$.)

TABLE 1.

THE EFFECT OF TIME OF EXPOSURE AND CONCENTRATION OF THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 1).

Time of Exposure.			Concentration of the Host Factor (Arbitrary Units).			
			100	50	25	12.5
2 Hours	..	..	93.5	90.1	72.2	49.8
4 "	..	..	94.6	90.0	89.6	69.7
6 "	..	..	90.8	91.6	92.2	86.0
8 "	..	..	90.1	88.9	88.2	89.2
10 "	..	..	92.0	90.8	91.8	89.5
3 Days	..	..	92.5	90.5	94.1	91.6

Least Significant Difference ($P = 0.05$): 3.5

TABLE 2.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST SOLUTION ON THE PERCENTAGE GERMINATION (EXPERIMENT 1).

(Mean Values for all Concentrations.)

Time of Exposure.	2 Hours.	4 Hours.	6 Hours.	8 Hours.	10 Hours.	3 Days.	Least Sig. Diff. ($P = 0.05$).
Mean Perc. Germ.	76.4	86.0	90.2	89.1	91.0	92.2	1.8

TABLE 3.

THE EFFECT OF CONCENTRATION OF THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 1).

(Mean Values for all Times of Exposure.)

Concentration of the Host Factor (Arbitrary Units).	100	50	25	12.5	Least Sig. Diff. ($P = 0.05$).
Mean Perc. Germ.	92.2	90.3	88.0	79.3	1.4

The results recorded in Table 2 show that the percentage germination increases as the time of exposure is lengthened from 2 hours to 6 hours,

but with a further extension of this factor the germination remains constant, maximal values being obtained throughout. The data of Table 3 indicate that an increased dilution of the host factor is accompanied by a decrease in the percentage germination.

The information to be gained from Table 1 can be summarized as follows:

(i) With concentrations of 100 and 50 units an exposure time of 2 hours suffices to produce maximal germination. With 25 units maximal germination is attained after an exposure of 4 hours, and with 12.5 units this effect is produced after an exposure of about 6 hours.

(ii) With an exposure time of 2 hours dilution has the greatest effect, the means for 25 and 12.5 units being significantly lower than those for 100 and 50 units. With an extension of the time of exposure the effect of dilution decreases until it is scarcely noticeable with an exposure time of 6 hours. Maximal germination is obtained with all concentrations of the stimulant after exposure periods of 8 hours, 10 hours and 3 days.

(b) *Experiment 2.*—The data of Tables 4–6 also show that the time of exposure and the concentration of the host factor both have a pronounced effect on the percentage germination.

TABLE 4.

THE EFFECT OF TIME OF EXPOSURE AND CONCENTRATION OF THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 2).

Time of Exposure.	Concentration of the Host Factor (Arbitrary Units).			
	100	50	25	12.5
15 minutes	53.1	45.1	22.8	10.9
30 "	72.3	57.0	34.4	20.6
45 "	82.2	67.4	43.0	30.5
3 days	92.3	91.1	90.0	87.9

Least Significant Difference ($P=0.05$): 4.2

TABLE 5.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 2).

(Mean Values for all Concentrations.)

Time of Exposure.	15 Mins.	30 Mins.	45 Mins.	3 Days.	Least Sig. Diff. ($P=0.05$).
Mean Perc. Germ. ..	33.0	46.1	55.8	90.3	2.1

TABLE 6.

THE EFFECT OF CONCENTRATION OF THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 2).

(Mean Values for all Times of Exposure.)

Concentration of the Host Factor (Arbitrary Units).	100	50	25	12.5	Least Sig. Diff. ($P=0.05$).
Mean Perc. Germ. . . .	75.0	65.2	47.6	37.5	2.1

From Table 4 it is evident that (i) with all concentrations of the stimulant all the means for time of exposure differ significantly from one another; and (ii) with exposure times up to 45 minutes all the means for concentration also differ significantly from one another, while in the case of the control (exposure time of 3 days) dilution has no appreciable effect, although the mean for 12.5 units is slightly lower than that for 100 units.

If the results of this experiment are compared with those of Experiment 1 it is clear that with short periods of exposure an extension of the time factor as well as a decrease in the concentration has a markedly more pronounced effect than with longer periods of exposure.

(2) *Experiments on the Effect of Time of Exposure in Relation to Time of Pre-exposure.*

(a) *Experiment 3.*—As regards the effect of time of exposure to the host stimulant the results of this experiment confirm the evidence obtained from Experiments 1 and 2, viz. that this factor has a highly significant effect on the percentage germination.

TABLE 7.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST FACTOR ON THE PERCENTAGE GERMINATION OF SEED PRE-EXPOSED FOR DIFFERENT PERIODS (EXPERIMENT 3).

Time of Exposure.	Time of Pre-exposure.		
	3 Days.	6 Days.	9 Days.
1 Hour	49.7	76.0	87.4
2 Hours	68.3	79.1	91.8
4 "	73.6	87.1	91.6
8 "	89.1	90.4	91.0
3 Days	88.5	91.6	92.5

Least Significant Difference ($P=0.05$): 4.5

TABLE 8.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 3).

(Mean Values for all Times of Pre-exposure.)

Time of Exposure.	1 Hour.	2 Hours.	4 Hours.	8 Hours.	3 Days.	Least Sig. Diff. (P=0.05).
Mean Perc. Germ.	71.1	79.7	84.1	90.2	90.9	2.6

TABLE 9.

THE EFFECT OF TIME OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION (EXPERIMENT 3).

(Mean Values for all Times of Exposure.)

Time of Pre-exposure (Days).	3	6	9	Least Sig. Diff. (P=0.05).
Mean Perc. Germ.	73.8	84.9	90.9	2.0

The data of Tables 7 and 9 also show that the time of pre-exposure likewise affects the germination in a very pronounced way. With an extension of the period of pre-exposure the percentage germination increases (Table 9).

The most outstanding feature of the results of Experiment 3 is the fact that the interaction between the two factors involved is also highly significant. (For each of the three components, time of exposure, time of pre-exposure and interaction, $P < 0.01$.) The nature of this interaction is indicated by the data of Table 7, it being evident that (i) as the period of pre-exposure is extended maximal germination is attained with progressively shorter times of exposure; and (ii) with a lengthening of the time of exposure maximal germination is obtained with progressively shorter periods of pre-exposure.

(b) *Experiment 4.*—The information to be gained from the results of this experiment is essentially the same as that from Experiment 3. The time of exposure and the time of pre-exposure both affect the percentage germination in a highly significant way, and these two factors are complementary in action. Nearly all the differences between the means in Table 10 are significant.

The means obtained for pre-exposure periods of 3 and 6 days combined with an exposure time of 3 days (Table 10) are lower than the corre-

TABLE 10.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST FACTOR ON THE PERCENTAGE GERMINATION OF SEED PRE-EXPOSED FOR DIFFERENT PERIODS (EXPERIMENT 4).

Time of Exposure.	Time of Pre-exposure.		
	3 Days.	6 Days.	9 Days.
15 Minutes	5.0	11.3	27.8
30 "	9.8	14.5	34.0
45 "	12.1	24.8	41.3
90 "	29.7	41.8	72.5
3 Days	44.0	60.1	89.7

Least Significant Difference ($P=0.05$): 3.7

TABLE 11.

THE EFFECT OF TIME OF EXPOSURE TO THE HOST FACTOR ON THE PERCENTAGE GERMINATION (EXPERIMENT 4).

(Mean Values for all Times of Pre-exposure.)

Time of Exposure.	15 Mins.	30 Min.	45 Mins.	90 Mins.	3 Days.	Least Sig. Diff. ($P=0.05$).
Mean Perc. Germ.	14.7	19.4	26.1	48.0	64.6	2.1

TABLE 12.

THE EFFECT OF TIME OF PRE-EXPOSURE ON THE PERCENTAGE GERMINATION (EXPERIMENT 4).

(Mean Values for all Times of Exposure.)

Time of Pre-exposure (Days).	3	6	9	Least Sig. Diff. ($P=0.05$).
Mean Perc. Germ.	21.2	30.5	53.1	1.6

sponding means in Table 7. Obviously the concentration of the host factor applied in Experiment 4 was lower than that used in Experiment 3. With this lower concentration of the stimulant the only mean that is more or less maximal is that for a pre-exposure period of 9 days combined with an exposure time of 3 days (Table 10).

DISCUSSION.

The results of this investigation indicate that when the seed is properly "sensitized", and a high concentration of the host factor is applied, a

relatively short time of exposure to the latter suffices to produce a high percentage germination. To germinate, the seed need not be in contact with the host solution during the entire second phase of germination. In Experiment 1 an exposure time of 2 hours to the undiluted host solution was sufficient to produce maximal germination in the "sensitized" seed (Table 1); in Experiment 3 more or less the same effect was obtained with a pre-exposure period of 9 days combined with an exposure time of 1 hour (Table 7); and in Experiment 2 more than 50 per cent of the "sensitized" seed germinated after an exposure of 15 minutes to the undiluted host solution (Table 4).

This evidence is in accordance with the results of Chabrolin (1939) and Brown and Edwards (1946) who investigated the germination of the seeds of *Orobanche speciosa* and *Striga lutea*, respectively. The former found that the action of the host factor on the "sensitized" seed is practically instantaneous. After only 30 seconds of immersion of this seed in an active decoction, followed by washing in running water for 10 minutes, the germination exceeded 60 per cent. He suggested that two substances which have a great affinity for one another are involved in the germination process. The one occurs in the "sensitized" seed and the other in the host solution. The latter penetrates very quickly into the living cells from which it cannot be removed by washing. Within the seed a chemical reaction takes place between these two substances (p. 126). To my mind, the idea that the pre-exposed seed contains a substance which is the same as or similar to that released from the host roots and that these two substances are complementary in action seems to be more feasible.

The results of Brown and Edwards (1946) indicate that a high percentage germination can also be obtained in pre-exposed seed of *Striga lutea* after relatively short periods of exposure to the host stimulant. With regard to the effect of time of exposure they distinguished between two phases, viz. an initial phase of superficial absorption of the host stimulant by the seed, followed by assimilation into the seedling. They found that with exposure to the host solution for 2, 4, 6 and 24 hours the effect of dilution of the host factor is, relatively, always the same, and that only in certain cases is there an increase in the germination as the period of exposure is increased. These observations were taken by them to indicate that absorption by the seed of the stimulant from the solution is complete within 2 hours. From my experiments no evidence could be obtained that in the case of *A. Vogelii* absorption is always complete within such a short period. Indeed, the results of Experiment 1 (Table 1) indicate that at 30° C. and with low concentrations of the host stimulant absorption may continue up to 6 hours or even longer.

It is, however, difficult to base any conclusions on this comparison of data, especially since it is not certain at what temperature exposure was carried out in the experiments of Brown and Edwards.

The interaction between time of exposure and concentration of the host factor, as observed in Experiments 1 and 2 of the present investigation, is quite comprehensible. Essentially the effect of only one factor was involved in these experiments, viz. that of the amount of the host stimulant absorbed by the seed. With an increase in the time of exposure the amount absorbed increases; and with an increase in the concentration of the stimulant in the host solution absorption is accelerated. This explains why (i) with an extension of the time of exposure progressively lower concentrations of the stimulant need be applied to produce maximal germination; and (ii) with an increase in the concentration of the stimulant the same effect can be obtained with progressively shorter times of exposure (Table 1).

As regards the interpretation of the results of Experiments 3 and 4 there is more uncertainty. Clearly the two factors involved, namely time of pre-exposure to moist conditions and time of exposure to the host solution, are also complementary in action. The most plausible explanation of this seems to be the hypothesis put forward by Brown and Edwards (1946) and already referred to above, viz. that during pre-exposure the seed itself synthesizes a substance which is the same as or similar to the stimulating substance released from the host roots. This hypothesis implies that during pre-exposure the amount of the substance formed in the seed itself gradually increases. And since the evidence of the present investigation indicates that the amount of the host stimulant absorbed by the seed increases with a lengthening of the time of exposure up to a certain point, it becomes clear that: (i) With an extension of the period of pre-exposure maximal germination can be obtained with progressively shorter times of exposure; and (ii) with a lengthening of the time of exposure the same effect can be produced with progressively shorter periods of pre-exposure.

SUMMARY.

1. Four two-factor experiments were carried out to study the effect of time of exposure to the host factor on the germination of the seed of *Alectra Vogelii*. In Experiments 1 and 2 the influence of this factor was investigated in relation to different concentrations of the stimulant; and in Experiments 3 and 4 its effect was studied in relation to various periods of pre-exposure of the seed to moist conditions.

2. In Experiments 1 and 2 it was found that the percentage germination increases with an increase in the time of exposure as well as with

an increase in the concentration of the host factor. The interaction between these two factors is highly significant. With long periods of exposure lower concentrations of the host factor are required to produce a high percentage germination than with short periods; and with high concentrations of the stimulant shorter periods of exposure are necessary to obtain the same effect than with low concentrations.

3. The results of Experiments 3 and 4 indicate that the factors, time of pre-exposure to moist conditions and time of exposure to the stimulant, are also complementary in action. With long periods of pre-exposure relatively short times of exposure are required to produce maximal germination; and with long times of exposure maximal germination can also be obtained after relatively short periods of pre-exposure.

4. These findings are consistent with the hypothesis that during pre-exposure the seed itself produces an active substance which is the same as or similar to that released from the host roots.

PART IV.

THE PROPERTIES AND PHYSIOLOGICAL SIGNIFICANCE OF
THE HOST FACTOR NECESSARY FOR THE GERMINATION OF
THE SEED OF *ALECTRA VOGELII* BENTH.

INTRODUCTION.

In a previous paper (Botha, 1948) preliminary results were presented which indicate that the host factor necessary for the germination of the seed of *Alectra Vogelii* is an unstable substance. When fresh host solutions were exposed to 60°C. and above they became less active within a relatively short time. At boiling point such solutions were totally inactivated within 10 minutes. When aged at room temperature in unstoppered flasks they retained their activity for longer periods, total inactivation occurring after from 4 to 8 days.

During the present investigation the instability of this substance was studied in more detail, particularly for the purpose of gaining more knowledge about its physical properties, chemical nature and physiological significance. Two aspects of this problem were investigated, viz. (a) the effect of temperature on the stability of the substance; and (b) the cause(s) of the instability of the substance at temperatures which correspond more or less to those prevailing under natural summer conditions. With regard to (b) several possibilities, which have already been enumerated previously (Botha, 1948), may be considered, the most important being:

- (1) The substance may be of a volatile nature and may consequently escape from solution even at relatively low temperatures.
- (2) The substance may be liable to oxidation by atmospheric oxygen.
- (3) The substance may be sensitive to light.
- (4) The substance may be decomposed by micro-organisms.
- (5) Like certain other active substances, the substance may undergo other chemical changes, such as changes through isomerization and denaturation.

Most of these possibilities were investigated experimentally. With regard to those mentioned under (1), (2) and (3), the results were entirely negative, thereby indicating that the substance is obviously not of a volatile nature and that atmospheric oxygen and light have no appreciable effect on its stability. For the sake of brevity I shall not discuss the experiments carried out in this connection, but shall confine myself more particularly to the effect of micro-organisms on the stability of the host factor.

A further aspect of the present investigation was concerned with the

possibility of the host factor being identical with, or similar in action to some active substance or other which is known to occur in plants. Since the host stimulant is definitely soluble in water, substances insoluble in water, e.g. the fat soluble vitamins, could be excluded. The possibility of its being identical with any of the following substances was investigated: aneurine (thiamin), riboflavin, nicotinic acid amide, ascorbic acid (vitamin C), pyridoxine and heteroauxin.

EXPERIMENTAL METHODS.

The host solutions were obtained from young cowpea plants cultured in distilled water. The details of obtaining these solutions and of the procedure of the germination tests were essentially the same as those given in the first paper of this series (Botha, 1950, I). In all experiments the *Alectra* seed was pre-exposed to moisture for 10 days at 30°C. before being used in the germination tests. All germination tests were replicated six times and the results were analysed for variance where necessary.

Experiment 1.—The effect of high temperatures (40–97°C.) on the stability of the host factor was investigated in this experiment. A fresh host solution was divided into several aliquot portions. Each portion was quickly heated to the desired temperature, kept at this temperature for the desired period of time and then quickly cooled in ice-cold water. To prevent evaporation, the flasks in which the solutions were heated, were furnished with reflux condensers. After the solutions had been cooled, their activity was tested on the pre-exposed seed.

Experiment 2.—In this experiment the effect of lower temperatures was studied. A fresh host solution was divided into four aliquot portions and stored in dark stoppered flasks at four different temperatures (30°C., 20°C., 10°C. and 0.5°C.). At certain intervals germination tests were carried out with small quantities of each solution.

Experiments 3 and 4.—In these two experiments the effect of micro-organisms on the stability of the host factor at room temperature and at 30°C. was investigated. Before these experiments could, however, be undertaken, several preliminary experiments had to be carried out in order to devise a convenient method for sterilizing or disinfecting the host solutions. Since the host factor is thermolabile, it was thought that sterilization by heat would probably not suit the purpose and recourse was therefore taken to chemical methods of disinfection. The suitability of various disinfectants, such as chloroform, ether, "Dettol," and silver nitrate, was tested. Of these, the last was found to be the most convenient. Silver nitrate has the advantage that the poisonous principle, silver, can easily be removed from the test solution before the germination tests are carried out. The effect of various concentrations of this dis-

infectant was also studied in preliminary experiments and it was found that a 1 per cent solution suited the purpose satisfactorily.

Each of the two main experiments (Experiments 3 and 4) was carried out as follows: A fresh host solution was divided into two aliquot portions. An appropriate amount of silver nitrate to obtain a 1 per cent solution of this disinfectant was added to the one portion (the test solution). No disinfectant was added to the other portion (the control solution).

The solutions were stored in dark stoppered flasks and at certain intervals small quantities of both were removed to carry out germination tests. In Experiment 3 the solutions were stored at room temperature, whereas in Experiment 4 they were stored in the incubator at 30°C.

Immediately before the germination tests were carried out, the removed solution samples were prepared as follows:

(a) *Test Solution*.—To 12.0 ml. of the test solution was added 3.5 ml. of a potassium chloride solution. The added amount of the latter solution contained slightly more than the amount of chloride necessary to precipitate all the silver in the 12.0 ml. of the test solution. The resulting silver chloride precipitate was filtered off, dry apparatus being used for filtering. The activity of the filtrate was then tested on pre-exposed *Alectra* seed.

(b) *Control Solution*.—From the procedure described under (a) it is clear that the addition of the potassium chloride solution to the test solution brought about a decrease in the concentration of the host factor; and that the filtrate contained potassium nitrate, since potassium chloride reacts with silver nitrate to form potassium nitrate. Both of these factors may affect the germination and this possibility was taken into consideration in preparing the control solution. To a first quantity of 12.0 ml. of the latter solution 3.5 ml. of a potassium nitrate solution was added. The amount of potassium nitrate added was equal to the amount of this substance formed in the test solution. The solution obtained in this manner is referred to as Control Solution 1.

A second quantity of 12.0 ml. of the control solution was diluted with 3.5 ml. of distilled water. The solution thus obtained, is called Control Solution 2.

From the above it is clear that the prepared test solution (filtrate of treatment under (a)) and Control Solution 1 contained the same concentration of potassium nitrate and that both solutions were diluted to the same extent. Control Solution 2 was also diluted to this extent but contained no potassium nitrate. From a comparison of the results obtained with Control Solutions 1 and 2 in the germination tests, it should therefore be possible to determine whether the presence of the

KNO_3 had an effect on the germination; and by comparing the data obtained with the test solution with those obtained with the two control solutions, it should be possible to determine the effect of disinfection on the stability of the host factor, provided that the effect of the KNO_3 was not decisive and that the host factor was not adsorbed by the silver chloride precipitate.

Experiments 5 and 6.—The effect on the germination of the *Alectra* seed of the active substances already mentioned was studied in these experiments. The sources of the substances applied were the following: for vitamin C—pure ascorbic acid; for "Vitamin B Complex"—"Benerva" tablets (Roche) each of which contained 1 mg. aneurine, 1 mg. riboflavin and 15 mg. nicotinic acid amide; for pyridoxine hydrochloride—"Hexabetalin" tablets (Lilly); and for heteroauxin—pure indole-3-acetic acid.

In order to obtain solutions of these substances, they were dissolved in aliquot portions of host solution and in distilled water. In the case of "Vitamin B Complex" and pyridoxine the tablets were first levigated, the appropriate amount of host solution or distilled water being added and then the insoluble ingredients were removed by filtration.

In Experiment 5 separate solutions of "Vitamin B Complex," vitamin C and heteroauxin were applied and in Experiment 6 each test solution contained a mixture of all the active substances mentioned above. The concentrations applied are given in Tables 5 and 6. These concentrations, as well as that of the host factor, were relatively high in Experiment 5, whereas in Experiment 6 low concentrations were applied. Since a fairly high percentage germination was obtained with a crude concentrate of the host factor at a dilution of 1 : 1,000,000 (Botha, unpublished work), these low concentrations were considered adequate for the purpose of the present investigation.

RESULTS.

1. *The Effect of High Temperatures on the Stability of the Host Factor (Experiment 1).*

The results of Experiment 1, which are recorded in Table 1, definitely indicate that the host factor is thermolabile. There was no appreciable decrease in the percentage germination induced by the host solutions which were pre-heated at 40°C., 50°C. and 60°C. for periods of up to 45 minutes. This also applied to pre-heating at 70°C. for periods of 15 and 30 minutes, but a slight decrease in activity was evident in the solution which was exposed for 45 minutes at this temperature. After exposure to 80°C., 90°C. and 97°C. (boiling point) there was a very pronounced decrease in the activity of the host factor. At boiling point it was practically completely inactivated within 15 minutes.

TABLE 1.

THE EFFECT OF HIGH TEMPERATURES ON THE ACTIVITY OF THE HOST FACTOR AS REFLECTED BY THE PERCENTAGE GERMINATION.

Temperature to which Heated (°C.).	Duration of Heating.		
	15 minutes.	30 minutes.	45 minutes.
40	86.6±1.9	89.1±2.0	88.4±0.6
50	85.5±1.3	90.0±1.8	87.5±1.5
60	83.5±1.7	87.6±1.6	85.3±2.2
70	89.1±1.8	91.6±1.8	77.5±1.8
80	61.8±3.7	5.8±1.3	1.3±0.7
90	28.4±2.2	0.7±0.7	3.1±1.3
97 (boiling point)	1.5±0.9	0.0	0.0

Control (solution not heated): 86.7±0.5

2. *The Effect of Temperatures of 30°C. and below on the Stability of the Host Factor (Experiment 2).*

The results of the second experiment are given in Table 2. These data show that the stability of the host factor increases with a fall in

TABLE 2.

THE EFFECT OF AGEING AT DIFFERENT TEMPERATURES ON THE ACTIVITY OF THE HOST SOLUTION AS REFLECTED BY THE PERCENTAGE GERMINATION.

Age of Solution in Days.	Temperature at which Solution was Stored.			
	0.5°C.	10°C.	20°C.	30°C.
2	98.2±2.4	92.3±1.4	96.1±1.5	96.5±0.9
4	94.2±1.1	96.3±1.5	93.4±1.4	69.7±4.8
6	97.1±0.7	92.9±2.5	56.0±4.5	27.7±3.5
8	96.7±1.0	80.8±6.2	31.6±6.1	15.5±3.5
10	96.6±0.5	52.9±3.6	16.7±3.7	18.7±8.3
12	95.3±1.1	23.5±6.6	2.4±1.0	0.7±0.4
14	94.5±1.1	16.4±4.6	1.4±0.6	1.8±1.0
18	97.2±1.4	8.6±4.9	0.6±0.6	1.0±0.6
27	95.6±1.7	0.0	0.0	0.0
41	95.3±2.4	—	—	—
99	89.0±1.8	—	—	—

Controls: 1. Fresh Solution: 96.5±1.0

2. Distilled Water: 1.3±0.7

temperature. The first decrease in its activity was evident after storage for 4 days at 30°C., after 6 days at 20°C. and after 8 days at 10°C., and inactivation was practically complete after 12 days at 30°C. and 20°C. and after from 18 to 27 days at 10°C. Germination could not be induced with solutions stored at 30°C., 20°C. and 10°C. when they were 27 days

old and the experiment was therefore terminated at this stage as far as these temperatures were concerned. Since the solution stored at 0.5°C . was, however, still fully active at this time, it was left in the refrigerator for further observations. When this solution was 41 days old, its activity still showed no decline and after storage for 99 days it still induced germination in about 90% of the seeds.

From these observations it is clear that the host stimulant retains its activity for months when stored at a temperature slightly above freezing point. This fact was very helpful in simplifying the germination technique for *A. Vogelii*, since in many types of experiment it obviates the necessity of preparing a fresh host solution for each germination test, when a series of consecutive tests have to be carried out over relatively long periods.

3. *The Effect of Micro-Organisms on the Stability of the Host Factor (Experiments 3 and 4).*

The results of Experiment 3 are presented in Table 3. From a comparison of the data in the third and fourth columns of this table it is clear that in general the values obtained with Control Solution 1 agree rather closely with the corresponding values obtained with Control Solution 2. This indicates that the presence of potassium nitrate in the former solution did not appreciably affect the percentage germination. In both control solutions the first decrease in activity became evident after storage for 12 days. When these solutions were 21 days old, they were completely inactive. The disinfected test solution, however, retained its original activity for at least 15 days. After storage for 21 days there evidently was a slight decrease in the activity of this solution, but no further decline in activity occurred from this time until the experiment was terminated. When it was 57 days old, this solution could still induce germination in approximately 85% of the seeds. It is evident from these observations that the host factor is fairly stable in a properly disinfected solution when stored at room temperature.

The results of Experiment 4, which are given in Table 4, also show that the potassium nitrate had no appreciable effect on the germination, there being no significant differences between the corresponding values for Control Solutions 1 and 2. In this experiment a very marked decrease in the activity of the control solutions was already noticeable after storage for 8 days. When they were 17 days old, these solutions were nearly completely inactive. The disinfected test solution, on the other hand, retained its original activity for at least 8 days. After storage for 17 days, a decline in activity was, however, also evident in this solution,

TABLE 3.

THE EFFECT OF DISINFECTION WITH SILVER NITRATE ON THE STABILITY OF THE HOST FACTOR AS REFLECTED BY THE MEAN PERCENTAGE GERMINATION.
(Solutions stored at room temperature—mean minimum: 17.7°C.; mean maximum: 23.6°C.)

Age of Solution in Days.	Test Solution ⁽¹⁾ (Disinfected).	Control Solution 1. ⁽²⁾	Control Solution 2. ⁽³⁾
0 (fresh)	93.1	91.3	91.1
3	94.5	95.5	93.0
6	93.1	90.9	90.7
9	89.5	88.0	95.7
12	93.7	64.4	70.2
15	93.9	11.7	14.0
21	81.4	0.0	0.0
27	86.7	0.0	0.0
33	82.9	0.0	0.0
45	83.3	0.0	0.0
57	86.5	0.0	0.0

Least Significant Difference ($P=0.05$): 3.5

⁽¹⁾Test Solution: Filtrate of 12.0 ml. disinfected solution+3.5 ml. KCl solution.

⁽²⁾Control Solution 1: 12.0 ml. control solution+3.5 ml. KNO₃ solution.

⁽³⁾Control Solution 2: 12.0 ml. control solution+3.5 ml. distilled water.

TABLE 4.

THE EFFECT OF DISINFECTION WITH SILVER NITRATE ON THE STABILITY OF THE HOST FACTOR AS REFLECTED BY THE MEAN PERCENTAGE GERMINATION.
(Solutions stored at 30°C.)

Age of Solution in Days.	Test Solution* (Disinfected).	Control Solution 1.*	Control Solution 2.*
0 (fresh)	97.4	95.7	97.6
4	92.6	90.4	90.2
8	92.4	42.3	43.2
17	80.7	1.8	4.3
31	14.3	0.0	0.3

Least Significant Difference ($P=0.05$): 6.7

*Solutions treated as in Table 3.

and at the age of 31 days inactivation had progressed to a very advanced stage. It is therefore clear that disinfection with silver nitrate is not an efficient method for preserving the host factor in solution at 30°C., although it also has a pronounced retarding effect on the inactivation process at this temperature.

4. *The Effect of Various Active Substances on the Germination of Alectra Seed.*

From the results of Experiment 5, which are given in Table 5, it is

TABLE 5.

THE EFFECT OF "VITAMIN B COMPLEX," VITAMIN C AND HETEROAUXIN ON THE GERMINATION OF THE SEED OF *A. Vogelii* (EXPERIMENT 5).

Treatment.	Percentage Germination.
1. "Vitamin B Complex" in Host Solution*	30.4 ± 18.6
2. "Vitamin B Complex" in Distilled Water*	0.0
3. 50 mg. Ascorbic Acid/100 ml. Host Solution	87.7 ± 1.4
4. 50 mg. Ascorbic Acid/100 ml. Distilled Water	0.0
5. 1 mg. Heteroauxin/100 ml. Host Solution	94.0 ± 1.5
6. 1 mg. Heteroauxin/100 ml. Distilled Water	0.0
7. Host Solution	87.1 ± 3.7
8. Distilled Water	0.0

*4 mg. aneurine, 4 mg. riboflavin and 60 mg. nicotinic acid amide per 100 ml. solvent.

clear that none of the active substances in question could induce germination in the seed of the parasite. The presence of ascorbic acid and heteroauxin in the host solution did not appreciably affect the germination brought about by the natural stimulant. The presence of "Vitamin B Complex" did, however, seem to have a detrimental effect on the germination. This may be due to an inhibitory action either of the active substances themselves, or of other soluble ingredients of the tablets used.

The results presented in Table 6 indicate that the combination of

TABLE 6.

THE EFFECT OF MIXTURES* OF "VITAMIN B COMPLEX," VITAMIN C, PYRIDOXINE HYDROCHLORIDE AND HETEROAUXIN ON THE GERMINATION OF THE SEED OF *Alectra Vogelii* (EXPERIMENT 6).

Treatment.	Percentage Germination.
1. Mixture 1 dissolved in Host Solution	30.1 ± 2.8
2. Mixture 1 dissolved in Distilled Water	0.0
3. Mixture 2 dissolved in Host Solution	43.4 ± 6.1
4. Mixture 2 dissolved in Distilled Water	0.0
5. Mixture 3 dissolved in Host Solution	35.4 ± 5.9
6. Mixture 3 dissolved in Distilled Water	0.0
7. Mixture 4 dissolved in Host Solution	43.6 ± 3.8
8. Mixture 4 dissolved in Distilled Water	0.0
9. Host Solution	51.8 ± 5.8
10. Distilled Water	0.0

*Ingredients of Mixtures.

(a) Mixture 1: 1 mg. aneurine, 1 mg. riboflavin, 15 mg. nicotinic acid amide, 2 mg. ascorbic acid, 2 mg. pyridoxine hydrochloride and 2 mg. heteroauxin per litre solvent.

(b) Mixtures 2, 3 and 4: The same ingredients, but concentrations respectively 25%, 10% and 1% of that in Mixture 1.

active substances applied in Experiment 6 did likewise not cause germination of the seeds. When applied in the presence of the host factor, this combination had an inhibitory effect.

DISCUSSION.

With the present investigation definite evidence has been obtained that the host factor of *A. Vogelii* is an unstable substance. At a temperature slightly above freezing point solutions of this substance can be stored for long periods without losing their activity, but with a rise in temperature, the rate of inactivation increases. At temperatures of 70°C. and above a decrease in activity occurs within a relatively short time.

At room temperature the instability of the substance in unsterilized solutions is due almost exclusively to decomposition by micro-organisms. The effect of micro-organisms is also clearly noticeable at 30°C., but at this temperature other chemical changes, which are as yet unknown, evidently occur as well.

The negative results obtained in Experiments 5 and 6 indicate that the host factor is obviously not identical with any of the following active substances: thiamin, riboflavin, nicotinic acid amide, ascorbic acid (vitamin C), pyridoxine and heteroauxin. In this connection it must, however, be borne in mind that the host stimulant may not be a single substance, but may be a complex of various factors acting in unison and constituting what is called by Schopfer (1943) a *constellation*. If this is so, it is clear that the mixture of substances applied in Experiment 6 (Table 6) evidently did not contain all the constituents of this constellation. The mixture might have contained some of the constituents, but it might just as well be that the host stimulant is composed of an entirely different set of factors.

Bearing in mind the abovementioned and other properties of the host stimulant, I shall now endeavour to elucidate its relation to other active substances occurring in plants.

The active substances ("Wirkstoffe" or *ergines*, according to Ammon and Dirscherl, 1948) are usually divided into three groups, viz. enzymes, hormones and vitamins. The question now arises whether the host stimulant of *A. Vogelii* belongs to any of these three categories. In endeavouring to decide this question, it will be necessary to give in outline the criteria for distinguishing between the three.

The enzymes can be distinguished from the other two groups on account of the fact that they are either proteins or proteids and are consequently colloidal substances with relatively large molecules. Like other proteinaceous substances, they are usually thermolabile and undergo denaturation when stored in solution. Being colloids, they are usually

not dialisable. Although there are many interrelations between enzymes, hormones and vitamins and although, according to the "Träger" theory, vitamins and hormones may actually form part of the constitution of active enzymes, the trained physiologist can usually decide with a fair amount of certainty whether a substance is an enzyme, or not.

From the botanical point of view it is, however, often extremely difficult to distinguish between hormones and vitamins. Several authors have already stressed this difficulty. Schlenker (1937, p. 79) states: "Es ist neuerdings bezweifelt worden, ob die Unterscheidung zwischen Hormonen und Vitaminen in der tierischen Physiologie noch Berechtigung hat, in der pflanzlichen Physiologie ist sie sicher nicht sinnvoll." Von Euler (1938) also realized this difficulty and proposed the term *ergone* to be used for hormones and vitamins in all cases in which it is not easy to distinguish between these two. More recently Schopfer (1943 and 1949) based a distinction between the two on their mode of action. He considers an action as hormonal if the substance in question acts, in the final analysis, specifically on the morphology of the organism. An action of vitamin nature is, on the other hand, according to him concerned with the intermediate metabolism and does not, in the final analysis, affect the morphology of the organism in a specific manner. He does, however, admit that even in his system, some substances are difficult to classify.

On account of this uncertainty with regard to the terminology, it is difficult to decide what place the host stimulant of *A. Vogelii* is to take among the active substances. This substance has the following two properties in common with enzymes: (a) it is thermolabile; and (b) apart from the fact that it can be decomposed by micro-organisms, it obviously also undergoes slow chemical changes when stored in solution at warm temperatures. It must, however, be stressed that these properties by no means guarantee that the substance is an enzyme. With regard to (a) it must be pointed out that, although inactivation by heat is in most cases so characteristic of enzymes that it is still at present used as a criterion for distinction, many authorities, e.g. Ammon and Dirscherl (1948), rightly consider this test as unreliable. And with regard to (b) it must be borne in mind that some active substances which clearly do not belong to the enzymes, also undergo chemical changes when stored. Kögl, Koningsberger and Erxleben (1936) have, for instance, found that pure auxin becomes physiologically inactive within a few months, even when sealed *in vacuo* and stored in the dark.

Much importance can, therefore, not be attached to these similarities between the host stimulant and enzymes. Indeed, some of my unpublished results are not consistent with the possibility of the substance being an

enzyme. It has, for instance, been found that the substance can pass through collodion and animal membranes and is therefore obviously a crystalloid (Botha, unpublished). The fact that the substance is absorbed by the seed material of *A. Vogelii* in physiologically active quantities within a relatively short time (Botha, 1951, III, above) may also be taken to indicate that it is probably not a colloid.

The second possibility, viz. that the stimulant may be a hormone, will now be considered. Since two of the well known phytohormones, viz. auxin A and auxin B, are thermostable, it is clear that the stimulant is not identical with these two substances. The possibility of its being identical with the so-called "rhizocaline" of Bouillenne and Went (1933) must be excluded for the same reason. As already mentioned, the results of the present investigation show that it also is probably not identical with heteroauxin. From these considerations it may be concluded that, if the substance is a phytohormone, it evidently belongs to a group of hormones which is as yet unknown.

In this connection the recent work of Brown and his collaborators (Brown, Johnson, Robinson and Todd, 1949; and Brown, Robinson and Johnson, 1949) is particularly interesting. According to them the stimulant necessary for the germination of the seed of *Striga hermonthica* only acts at a comparatively late stage in the germination process, and then it has the effect of promoting the extension of the cells of the radicle. They have found that this stimulant also promotes cell extension in segments of pea-roots. This effect might clearly be considered as hormonal. Although they did not succeed in isolating the stimulant in the pure state, they have found that concentrates of the host root exudations contain pentose sugars which are probably not aldopentoses, and that a simple sugar, viz. D-xyloketose, also induces germination in the seed of the parasite. The effect of this sugar on segments of pea-roots is, according to their results, similar to that of the natural stimulant. They conclude that the activity of their concentrates was due to a substance which is either very similar to or identical with D-xyloketose. Furthermore, they rightly point out that theirs is the first record of a stimulating effect on the growth of plant tissues of a simple sugar in concentrations of less than 10 mg./litre.

The third possibility, viz. that the host stimulant of *A. Vogelii* may be a vitamin, also seems to be quite feasible. The mere fact that the parasite requires an active substance from an external source for the germination of its seed, suggests that it has lost the capability of synthesizing some substance or other which is essential for germination. Such a loss of the capacity to synthesize essential active substances is by no means uncommon in plants. Schopfer (1943) cites many examples of

lower plants in which it occurs and also points out that it is met with in higher plants as well. In discussing the work of Burgeff (1936) and others on the germination of the seeds of orchids with endotrophic mycorrhiza, he (i.e., p. 251) states: "Thus the problem is placed in its true perspective when we say that, as in the case of auxo-heterotrophic micro-organisms, the orchid embryo suffers an avitaminosis which can be compensated by supplying the medium with the missing factor which the plant is no longer able to synthesize."

When the results of Burgeff (1936) on the germination of the seeds of certain orchid genera are compared with the available data on the germination of the seeds of angiospermous root-parasites, a striking similarity in essential points is evident. Like the orchid seed, the seed of the parasite probably suffers an "avitaminosis." To my mind the loss of the capacity to synthesize the substance necessary for germination is, however, not complete in the case of the parasite. During pre-exposure to moist conditions an inadequate amount of this substance is formed in the seed and this amount has to be supplemented with the host factor before germination can take place. The definition of a vitamin in its more or less classical sense is indeed applicable to the host stimulant in so far as it is synthesized in one organism (the host) and has a catalytic action in another (the parasite). Since there is, however, reason to believe that this substance induces cell extension in the radicle of the parasite, it may, on the other hand, be considered as hormonal in its mode of action.

In view of all these considerations I am of the opinion that, until further information on its properties and mode of action is available, it will be safest to classify the host stimulant of *A. Vogelii* under von Euler's (1938) ergones (see above). To my mind the host factors of various other angiospermous root-parasites which have been studied hitherto, also belong to this group of active substances, e.g. that of *Striga lutea* (studied by Saunders, 1933; and Brown and Edwards, 1944 and 1946), that of *Striga hermonthica* (studied by Andrews, 1945; Brown and his co-workers (see above); and Vallance, 1950 and 1951) and that of *Orobanche speciosa* (studied by Chabrolin, 1939).

In order to distinguish the host stimulants of the angiospermous root-parasites from other ergones, it is, to my mind, in any case necessary to introduce a special term. I propose the term *parasitoblastin** for this purpose. A parasitoblastin may be defined as an organic substance or a

*On account of its possible role in germination Cholodny (1935) proposed the term *blastanin* for the hormone produced in the endosperm of the *Avena* seed. Went and Thimann (1937, p. 63) pointed out that, since the activity of this substance was determined on *Avena*, it falls under the definition of auxin. Cholodny's term is, therefore, obviously redundant.

group of organic substances which is released by the roots of non-parasitic flowering plants and which, when present in minute quantities, induces germination in the seed of some angiospermous root-parasite or other. To avoid misunderstanding, I particularly wish to stress the possibility that a parasitoblastin might not be a single substance but might be composed of two or more factors acting in unison and supplementing certain germination ergones which the parasite is no longer able to synthesize in sufficient quantities.

The host-dependent germination of the seeds of angiospermous root-parasites must obviously be explained as follows: In their adaptation to the parasitic mode of life these plants have, to a great extent, lost the capacity to synthesize certain ergones which are essential for the germination of their seeds. These or similar ergones, viz. the parasitoblastins, they now obtain from the host roots. This idea may serve as a working hypothesis for further research in this direction.

SUMMARY.

1. Several experiments were carried out to study the effect of temperature and of disinfection on the stability of the host factor necessary for the germination of the seed of *A. Vogelii*.

2. It was found that the stimulant is thermolabile, inactivation occurring within a relatively short time at temperatures of 70°C. and above. At a temperature slightly above freezing point solutions of the substance can be stored for long periods without losing their activity, but as the temperature rises, the rate of inactivation increases.

3. At room temperature (18—24°C.) the instability of the substance in unsterilized solutions is almost exclusively due to decomposition by micro-organisms. The effect of micro-organisms is also clearly noticeable at 30°C., but at this temperature other, unknown chemical changes evidently occur as well.

4. Experiments were also carried out to determine whether the host factor is identical with some active substance or other which is known to occur in plants. The results obtained, indicate that the stimulant is probably not identical with any of the following substances: Auxin A, auxin B, heteroauxin, rhizocaline, thiamin, riboflavin, nicotinic acid amide, pyridoxine and vitamin C.

5. The possible relation of the stimulant to other active substances is discussed and it is concluded that it must evidently be classified with von Euler's *ergones*.

6. The term *parasitoblastins* is suggested for the host factors necessary for the germination of the seeds of angiospermous root-parasites.

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